simultaneously extending said at least three pairs of annealed primers from each primer's 3′ terminus to synthesize an extension product complementary to the strands annealed to each primer, said extension products, after separation from their complement, being capable of serving as templates for the synthesis of an extension product from the other primer of each pair;

separating said extension products from said templates to produce single-stranded molecules;

amplifying said single stranded molecules by repeating, at least once, said annealing, extending and separating steps; and

identifying said amplified extension products from each different sequence.

11 ~~20~~. (four times amended) A method for simultaneously detecting at least three DNA sequences, comprising the steps of:

adding to a common reaction vessel containing a sample mixture of at least three distinct, target sequences in single-stranded form, at least three pairs of oligonucleotide primers, each pair specific for a different sequence, one primer of each pair substantially complementary to a part of the sequence in the sense-strand and the other primer of each pair substantially complementary to a different part of the same sequence in the complementary anti-sense strand; [wherein the primers all have similar melt temperature characteristics;]

annealing the at least three paris of primers to their complementary sequences, all primers being subject to the same reaction conditions;

simultaneously extending said at least three pairs of annealed primers from each primer's 3' terminus to synthesize an extension product complementary to the strands annealed to each primer, said extension products, after separation from their complement, being capable of serving as templates for the synthesis of an extension product from the other primer of each pair;

separating said extension products from said templates to produce single-stranded molecules;

amplifying said single stranded target sequences by repeating, at least once, said annealing, extending and separating steps; and

identifying whether amplified extension products have been synthesized from each different sequence, as a [measure] result of the presence or [amount] absence of each target sequence.

Please add claims 21-24.

21. A method for simultaneously detecting known deletions from at least three DNA sequences, comprising the steps of:

treating said DNA to form single-stranded complementary strands;

adding at least three pairs of oligonucleotide primers, each pair specific for a different sequence, one primer of each pair substantially complementary to a part of the sequence in the sense-strand and the other primer of each pair substantially complementary to a different part of the same sequence in the complementary anti-sense strand and each primer having a Tm such that the lowest Tm and highest Tm of all added primers varies by no more than 8.5° C;

annealing the at least three pairs of primers to their complementary sequences, all primers being subjected to the same reaction conditions;

simultaneously extending said at least three pairs of annealed primers from each primer's 3′ terminus to synthesize an extension product complementary to the strands annealed to each primer, said extension products, after separation from their complement, being capable of serving as templates for the synthesis of an extension product from the other primer of each pair;

separating said extension products from said templates to produce single-stranded molecules;

amplifying said single stranded molecules by repeating, at least once, said annealing, extending and separating steps; and

identifying said amplified extension products from each different sequence.

22. A method for simultaneously detecting at least three DNA sequences, comprising the steps of:

adding to a common reaction vessel containing a sample mixture of at least three distinct, target sequences in single-stranded form, at least three pairs of oligonucleotide primers, each pair specific for a different sequence, one primer of each pair substantially complementary to a part of the sequence in the sense-strand and the other primer of each pair substantially complementary to a different part of the same sequence in the complementary anti-sense strand and each primer having a Tm such that the lowest Tm and highest Tm of all added primers varies by no more than 8.5° C;

annealing the at least three paris of primers to their complementary sequences, all primers being subject to the same reaction conditions;

simultaneously extending said at least three pairs of annealed primers from each primer's 3' terminus to synthesize an extension product complementary to the strands annealed to each primer, said extension products, after separation from their complement, being capable of serving as templates for the synthesis of an extension product from the other primer of each pair;

separating said extension products from said templates to produce single-stranded molecules;

amplifying said single stranded target sequences by repeating, at least once, said annealing, extending and separating steps; and

identifying whether amplified extension products have been synthesized from each different sequence, as a result of the presence or absence of each target sequence.

23. A method for simultaneously detecting known deletions from at least three DNA sequences, comprising the steps of:

treating said DNA to form single-stranded complementary strands;

adding at least three pairs of oligonucleotide primers, each pair specific for a different sequence, one primer of each pair substantially complementary to a part of the sequence in the sense-strand and the other primer of each pair substantially complementary to a different part of the same sequence in

the complementary anti-sense strand and each primer having a Tm such that the lowest Tm and highest Tm of all added primers varies by no more than 4.5° C;

annealing the at least three pairs of primers to their complementary sequences, all primers being subjected to the same reaction conditions;

simultaneously extending said at least three pairs of annealed primers from each primer's 3′ terminus to synthesize an extension product complementary to the strands annealed to each primer, said extension products, after separation from their complement, being capable of serving as templates for the synthesis of an extension product from the other primer of each pair;

separating said extension products from said templates to produce single-stranded molecules;

amplifying said single stranded molecules by repeating, at least once, said annealing, extending and separating steps; and

identifying said amplified extension products from each different sequence.

15 24. A method for simultaneously detecting at least three DNA sequences, comprising the steps of:

13 cont. 1 8

adding to a common reaction vessel containing a sample mixture of at least three distinct, target sequences in single-stranded form, at least three pairs of oligonucleotide primers, each pair specific for a different sequence, one primer of each pair substantially complementary to a part of the sequence in the sense-strand and the other primer of each pair substantially complementary to a different part of the same sequence in the complementary anti-sense strand and each primer having a Tm such that the lowest Tm and highest Tm of all added primers varies by no more than 4.5° C;

annealing the at least three paris of primers to their complementary sequences, all primers being subject to the same reaction conditions;

simultaneously extending said at least three pairs of annealed primers from each primer's 3' terminus to synthesize an extension product complementary to the strands annealed to each primer, said extension

products, after separation from their complement, being capable of serving as templates for the synthesis of an extension product from the other primer of each pair;

separating said extension products from said templates to produce single-stranded molecules;

amplifying said single stranded target sequences by repeating, at least once, said annealing, extending and separating steps; and

identifying whether amplified extension products have been synthesized from each different sequence, as a result of the presence or absence of each target sequence.

Please contact the undersigned at (713) 651-5325 with any questions.

Respectfully submitted,

Date: 9/30/94

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